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Screening and characterization of nutrient solubilizing phytobeneficial rhizobacteria from healthy coconut palms in root (wilt) diseased tract of Kerala, India

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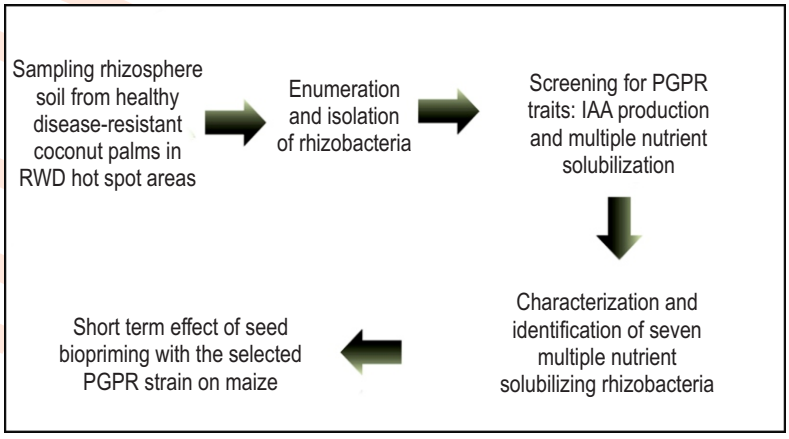
Abstract

Aim: Isolation, screening and characterization of beneficial rhizosphere bacteria associated with healthy (field-resistant) coconut palms in root (wilt) disease endemic hotspot areas of Kerala.

Methodology: One hundred and ten rhizobacterial isolates associated with healthy coconut palms of root (wilt) diseased tract of Kerala were isolated and screened *in-vitro* for IAA production and solubilization of fixed forms of mineral nutrients. Seven isolates showing multiple phytobeneficial properties were characterized and the selected isolate was tested for its biopriming effect on maize.

Results: Of the total isolates screened, 54 isolates produced IAA. Among the nutrient solubilizers, silicate solubilizers (57%) and phosphate solubilizers (48%) dominated. Of the seven isolates with multiple phytobeneficial properties, five rhizobacterial isolates belonged to Enterobacteriaceae family including three *Enterobacter* spp. The isolate T4HFB9 belonged to *Acinetobacter* sp.. The green fluorescent *Pseudomonas* isolate K3HPSB2, showed 99% sequence similarity with *Pseudomonas migulae*. Seed biopriming of maize with *Pseudomonas* sp. strain K3HPSB2 recorded significant increase in germination percentage and seedling vigour index over untreated control.



Interpretation: Disease-resistant coconut palms in RWD endemic tracts host a good proportion of phytobeneficial rhizosphere bacteria,with demonstrable multiple plant growth promoting traits. Multi-nutrient solubilizing *Pseudomonas* sp. with bioinoculant prospects has been selected for further studies on bio-priming for palm health management in RWD endemic tracts.

Key words: Coconut, Nutrient solubilizers, Phytobeneficial, *Pseudomonas*, Rhizobacteria

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Introduction

Root (wilt), a phytoplasmal disease of coconut, though non-lethal, is debilitative resulting in drastic reduction of yields and remains a major production constraint in southern districts of Kerala. Root (wilt) disease (RWD) was first reported from Kottayam district (1882) of Kerala in India. Since then, the disease has spread in all directions prevailing in eight out of 14 districts of Kerala in a contiguous manner. Disease spread extending to newer areas, especially the bordering districts of Tamil Nadu (Srinivasan *et al.,* 2000; Meena and Samiappan, 2012) and its occurrence in isolated pockets of south Karnataka and Goa are of serious concern. Affecting palms of all age groups, RWD exhibits external diagnostic symptoms such as flaccidity, yellowing and marginal necrosis of leaflets along with yield reduction and quality decline of oil, kernel and husks due to physiological and biochemical changes brought by the disease. As disease advances, rotting of roots, dropping of immature nuts and necrosis of spikelets are more pronounced thereby causing severe economic loss to the coconut growers. Leaf rot disease, caused by a fungal complex with destructive potential, is widely prevalent in RWD-affected coconut palms. There is no effective and economic therapeutic control or preventive measures available for the disease till date (Solomon *et al.,* 2018). Lack of control measures demands adoption of management strategies involving multidisciplinary approaches. Phytobenefical rhizobacteria that support host plants through mechanisms such as biostimulation, biofertilization and bioprotection, can form one of the components of disease management strategies.

Rhizo-microbial community composition and assemblages and their expressive functions are highly influenced by root metabolites that reflect the host plant health status. Selection pressure via root metabolites help plants to recruit favourable microbial partners from the inoculum pool adapted to the surrounding soil and environment (Dennis *et al.,* 2010). Nutrients and metabolites of root exudates offer energy and signals that stimulate the resident microbiota to promote their ecosystem functions such as nutrient acquisition and cycling. Resultant microbial networking and plant-microbiome interaction evolve into specialized mutualism (Long *et al.,* 2008), thereby host plant and microbiome become more beneficial for each other. Increasing evidences on core microbial community forming stable association with host plants across temporal and spatial scale and perturbations in microbiomes during disease progression suggest that disease establishment is associated with changes in the host associative microbiome (Lamichhane and Venturi, 2015). Direct benefits of host associative microbiota include facilitating acquisition of essential micronutrients, thereby enhancing nutrient use efficiency as well as imparting resilience to biotic and abiotic stress (Bargaz *et al.,* 2018). Dominant bacterial classes associated with healthy host plants are capable of biocontrol activity and /or inducing host defense.

High populations of bacterial genera such as *Pseudomonas*, *Bacillus, Burkholderia* and actinomycetes are often reported in soils with general disease suppressiveness. Metagenome analyses has proved association of Gammaproteo bacteria in relative abundances with healthy plant hosts in comparison to the diseased counterpart. Emerging studies on decrypting core microbiome associative of plant host have shown rhizo-microbiome providing complementary host services such as 'disease protection' and 'nutrient provision' (Mendes *et al.,* 2011; Trivedi *et al.,* 2012; Hartman *et al.,* 2017). Moreover, based on the knowledge emanated from plant-microbiome interaction studies, researchers have expressed that understanding natural phenomena of disease suppressiveness developed in soil would result in viable strategies for the management of plant health in rhizosphere shaping through transfer of bespoke core microbiome and stimulation of resident/inoculated beneficial microbial consortia (Gopal *et al.,* 2013; Kafle *et al.,* 2019).

Investigations on rhizosphere microflora of coconut palm indicate proliferation of beneficial microbial groups like nitrogen fixers and phosphate solubilizers in coconut rhizosphere under mixed cropping with fodder crops like hybrid napier and fodder legumes in the RWD affected region. Green manures are found to be more efficient in terms of yield and stimulation of beneficial microbial activities (Thomas and Shantaram, 1984). Observations on the adverse effect of coconut RWD on mycorrhizal symbiosis of coconuts and the superiority of intercropping systems in increasing the mycorrhizal status of RWD affected coconut palms have been recorded (Thomas, 1988). Previous studies on variations in beneficial rhizosphere microorganisms in RWD-diseased and field-resistant coconut palms reveal that the field-resistant palms host 3.6% of beneficial microorganisms, particularly silicate solubilizing bacteria and fungi, thus hypothesizing higher population of beneficial microflora promoting more nutrient uptake and improving soil health (Gopal *et al.,* 2005). Interventions with organic inputs including vermicompost have been found to improve the health of RWD palms and increase their nut yields (Krishnakumar and Maheswarappa, 2010). Possibly vermicompost application enriches plant-beneficial microbiota,altering the soil microbial structure and function thereby improving nutrient mineralization and root absorption leading to better soil and plant health (Gopal *et al.,* 2012).

All these studies recommend appropriate basin management practices and intercropping systems to increase nutrient availability and disease alleviation through enrichment of beneficial microorganisms in plantation crops.Having analyzed microbial diversity of palms infected and non-infected by Cote d'Ivoire lethal yellowing (CILY) phytoplasma, Morales-Lizcano *et al.* (2017) described microbial genera specific to symptomless palms, suggesting their prospects in improving the management of CILY. Despite their importance, limited are the studies on the characterization of dominant microbial strains with phytobeneficial properties associated with field-resistant coconut palms in RWD tract, which can refine interventions in RWD management through bioformulations of efficient microbial inoculants to improve palm health. In light of the above, this study

was carried out to characterize the plant growth promoting rhizosphere bacteria associated with root (wilt) disease free/field-resistant coconut palms located in root (wilt) disease hotspot areas of southern Kerala.

Materials and Methods

Sampling: Root samples along with rhizosphere soil were collected from healthy coconut palms located in the hotspot areas of RWD in Kottayam and Pathanamthitta districts of Kerala. Disease-resistant palms were selected based on visual symptoms following standard methods of disease indexing for RWD in coconut palms (George and Radha, 1973) and DAC indirect ELISA (Direct Antigen Coated Indirect Enzyme Linked Immunosorbent Assay) results that used RWD phytoplasma-specific antibody for rapid screening of coconut samples (Sasikala *et al.,* 2010). Other parameters like yield (>80 nuts/year/palm), age (30 - 40 years) and variety (West Coast Tall) were also considered for selecting palm. Soil samples from the representative palms were collected at a distance of 1 m from the trunk of palms to a depth of 30 cm from the surface. The samples were analyzed for pH, organic carbon and available nutrients (Jackson, 1973).

Isolation of rhizobacteria: General and function-specific microbial populations of coconut rhizosphere were estimated following standard serial dilution and plating technique (Alef, 1995). Dominant culturable bacteria were isolated on nutrient agar and on differential media such as King's B agar (for fluorescent pseudomonads) and Pikovskaya's agar (for phosphorus solubilizing bacteria). *Azospirillum* isolates were isolated from root bits in semisolid nitrogen-free malate medium (Baldani and Dobereiner, 1980). Rhizosphere bacterial isolates of different morphotypes obtained on general and selective media were purified. Hundred and ten isolates including eight *Azospirillum* isolates were maintained by successive sub-culturing on nutrient agar slants and stored at 4°C for further studies.

Screening rhizobacteria for plant beneficial traits: Indole acetic acid (IAA) production was quantitatively measured by the method of Gordon and Weber (1951). All the bacterial isolates were screened for phosphate solubilization on Pikovskaya's agar containing tricalcium phosphate as inorganic phosphate source (Pikovskaya, 1948). To screen for zinc solubilizing bacteria, broth cultures of bacterial isolates (10 µl of log-phase bacterial culture) were spot inoculated on the plates of mineral salts agar medium supplemented with 0.1% zinc oxide. Screening for silicate and potassium solubilization was done on glucose agar medium supplemented with 0.25% of magnesium tri silicate and Aleksandrow agar (HiMedia) containing 0.2% potassium alumino silicate, respectively. The plates were incubated at 30 ± 2°C for 10 days and observed for clearing zone around the bacterial growth. Solubilization indices (P, Zn, Si and K) of the selected isolates were calculated as the ratio of total diameter (colony + halo zone) and colony diameter in the respective media (Premono *et al.,* 1996).

Characterization of rhizobacterial isolates: Biochemical characterization of selected rhizobacterial isolates was done using suitable HiMedia kits (Hi25™ Enterobacteriaceae identification kit and Hi24™ Nonfermenters identification kit). Molecular characterization was done following 16S rRNA gene sequencing. Genomic DNA of bacteria was extracted from 24 hr old cultures grown in nutrient broth using QIAamp DNA Mini Kit (QIAGEN India (P) Ltd, New Delhi). PCR amplification was done using universal bacterial primers (F27- 5´-AGAGTTTGATCMTGGCTCAG-3´and R1492-5´-TACGGYTACCTTGTTACGACTT-3´) (Sambrook and Russel, 2001) with a few modifications and the sequencing was outsourced to Agrigenome Labs Pvt. Ltd., Kochi. Sequences were searched for homologous sequences at GenBank database using Basic Local Alignment Search Tool (BLAST) analysis at NCBI. Multiple sequence alignment was done using CLUSTAL X. The evolutionary history was inferred using the Neighbor-Joining method (Tamura *et al.,* 2004) and the distances were computed using Kimura 2-parameter method (Kimura, 1980). Phylogenetic tree was constructed in MEGA7.

Effect of *Pseudomonas* sp. K3HPSB2 on maize: Short term effect of seed biopriming with the selected PGPR strain *Pseudomonas* sp. K3HPSB2 that showed high mineral (multiple) solubilizing capacity was studied in maize to assess its growth promoting potential. Cell suspension of *Pseudomonas* sp. K3HPSB2 grown in nutrient broth (30+/-2°C) was collected after 24 hr of inoculation. Cells collected by centrifugation (8000 rpm for 5 min) were washed twice with sterile distilled water and the dilution was adjusted to 10^8cfu ml^{-1}, as confirmed by plate count method. Maize seeds (African Tall, TNAU) were surface sterilized with alcohol (70% for 1 min) followed by sodium hypochlorite (2.5% for 1 min) and rinsed three times with sterile distilled water. Seeds were treated by soaking in the diluted inocula of *Pseudomonas* sp. K3HPSB2 for one hour and then placed in moistened sterile coir pith media taken in sterile phyta jar. Seeds soaked in sterile distilled water were maintained for control. Growth parameters were recorded 7 days after sowing (DAS) to determine the seedling vigour index [(root length + shoot length) × germination percentage] (Abdul-Baki and Anderson, 1973).

Statistical analysis: Statistical analyses were performed with Excel (version 2010) and WASP 2.0 (Web Agri Stat Package 2.0). The data were subjected to analysis of variance and the differences among various treatment means were compared with critical difference (CD) value at 5% ($P \leq 0.05$) probability level.

Results and Discussion

To study the rhizosphere bacterial composition of RWD field resistant coconut palms, Kumarakom and North Kiliroor of Kottayam district and Manippuzha, Nedumburam and Thevery of Pathanamthitta districts were selected for sampling. These areas were earlier identified as hotspot areas of RWD of coconut.

Table 1: Population count of general and function-specific microbes in the rhizosphere of healthy/disease-resistant coconut palms.

	Microbial population (cfu g^{-1} dry soil)*					
	General			Function-specific		
District	Bacteria $\times 10^6$	Actinomycetes $\times 10^5$	Fungi $\times 10^3$	Fluorescent pseudomonads $\times 10^2$	Phospho-bacteria $\times 10^4$	Free-living nitrogen fixers $\times 10^5$
Kottayam	17.9±12.3 (7.25)	12.7±2.8 (6.10)	5.35±5.2 (3.73)	6.3±4.5 (2.80)	7.3±4.2 (4.86)	18.0±7.0 (6.26)
Pathanamthitta	9.5±4.8 (6.98)	24.2±5.9 (6.38)	6.1±5.5 (3.79)	3.5±3.41 (2.54)	2.8±1.2 (4.45)	20.7±11.3 (6.32)

*(Values in parentheses indicate log transformed values of average microbial count). Values are mean±S.D.

Table 2: Molecular and biochemical characteristics of selected PGPR

Isolate	GenBank Accession number of 16S rRNA sequence	Closest related species (% similarity)	Gram reaction	Catalase	Oxidase	Nitrate reduction	Citrate utilization	Urease	β-galactosidase	Growth in N-free medium
K1HPSB1	MK078042.1	*Klebsiella pneumoniae* strain DSM 30104^T (99.72%)	-	+	-	+	+	+	+	+
K3HPSB1	MK100337.1	*Enterobacter tabaci* strain YIM Hb-3^T (99.71%)	-	+	-	-	+	+	-	+
K3HPSB2	MK100338.1	*Pseudomonas migulae* strain CIP 105470^T (98.64%)	-	+	+	-	+	+	-	+
T4HFB9	MK092981.1	*Acinetobacter pitti strain DSM* 21653^T (99.86%)	-	+	-	+*	+	+	-	+
T4HFB11	MK100341.1	*Atlantibacter subterranea* strain FRC1^T (99.54%)	-	+	-	+	+	+	+	+
T2PSB3	MK093215.1	*Enterobacter tabaci* strain *YIM* Hb-3^T (99.57%)	-	+	-	+	+	+	+	+
T6PSB1	MK093216.1	*Enterobacter cloacae* strain ATCC 13047^T (99.43%)	-	+	-	+	+	+	+	+

* weakly positive

Disease-free palms in the root (wilt) endemic areas identified earlier were utilized for developing resistant varieties through long-term breeding strategies (Nair *et al.,* 2010). Coconut palms, three from Kottayam and four from Pathanamthitta districts, categorized as 'apparently healthy' with a disease index of '0' and that showed negative reaction in serodiagnostic test were selected for rhizosphere soil sampling. Soils were strongly acidic in reaction in Kottayam with a mean pH of 5.0 and was moderately acidic (6.2) in the observed sites of Pathanamthitta district. Organic carbon content ranged from 0.6 to 1.8% in the sampling sites. High amounts of available nutrients such as P, Ca, Fe, Mn, Cu and Zn were present in the soil samples with an average content of 35.85 ppm, 448.88 ppm, 87.37 ppm, 29.49 ppm, 4.25 ppm and 6.25 ppm, respectively. Sufficiency of available K (166 ppm) and Mg (128 ppm) was observed in the locality.

Rhizosphere soil samples from healthy palms showed good representations of general and function-specific bacteria (Table 1). The mean bacterial population was 1.3×10^7 cfu g^{-1} dry soil with no significant location wise variations. Notably higher population of actinomycetes and free-living nitrogen fixers to the tune of 2×10^6 cfu g^{-1} were observed irrespective of location. Wider variations within the locations can be attributed to differences in soil type (alluvial to laterite soil), cropping system and management practices that highly influenced the rhizosphere microbial composition. Higher population of function specific rhizosphere bacteria such as nitrogen fixers, phosphate and silicate solubilizers in field-tolerant palms in comparison with RWD palms has been reported by Gopal *et al.* (2005). Other systemic plant diseases were also known to restructure root associated bacterial communities revealing more bacterial diversity and abundance of phytobeneficial rhizobacteria and functional genes in uninfected host plants (Trivedi *et al.,* 2012). George *et al.* (2018) reported coconut palms harbouring varied populations of psuedomonads and *Bacillus* spp. with multiple plant growth promoting traits through strategic screening approaches.

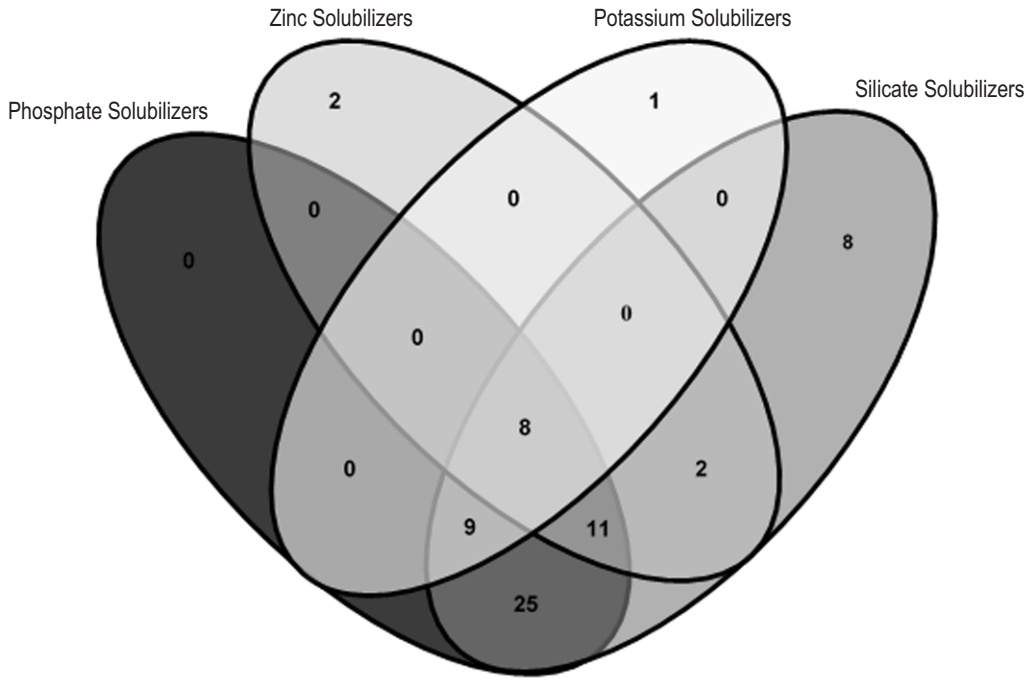

Fig.1: Venn diagram illustrating the number of PGPR with mineral nutrient solubilization potential.

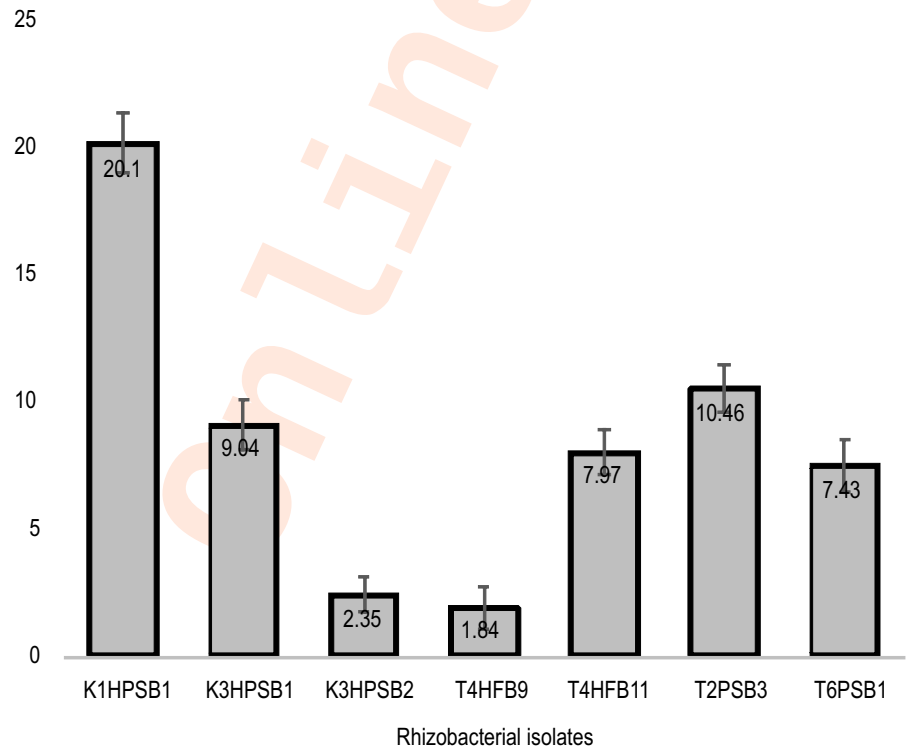

Fig. 2: Quantitative assay of IAA production by the rhizobacterial isolates screened for mineral nutrient solubilization. Error bars indicate standard error of mean (n=3).

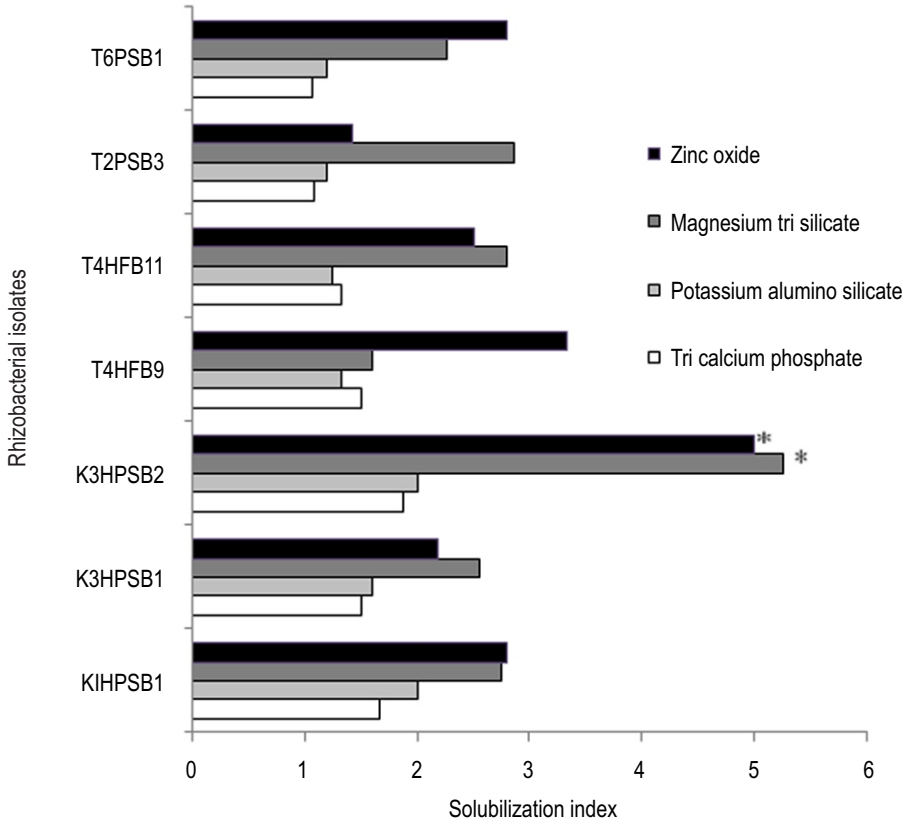

Fig. 3: Mineral nutrient solubilization efficiency of selected rhizobacteria. Values are mean of three replications. * significant at $p<0.05$.

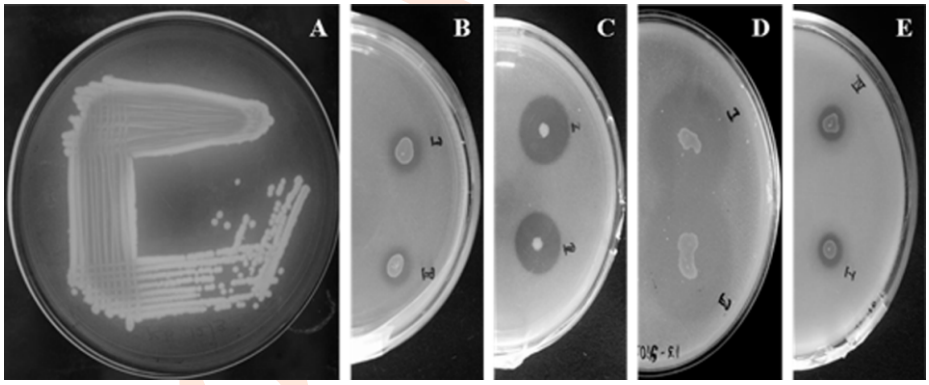

Fig. 4: (A) Multiple nutrient solubilization potential of fluorescent *Pseudomonas* sp. strain K3HPSB2; (B) Phosphate (Tricalcium phosphate) solubilization on Pikovskaya's agar medium; (C) Zinc (zinc oxide) solubilization on mineral salts agar; (D) Silicate (magnesium tri silicate) solubilization on glucose agar and (E) Potassium solubilization (potassium alumino silicate) on Aleksandrow agar.

IAA production was selected as prime PGP trait for screening as this property determines the colonising efficiency of rhizobacteria and, hence, determinative of its growth promotion potential. Of the total isolates, 54 tested positive for IAA production ranging from 1 to 20 µg ml^{-1} of culture filtrate. Among the IAA producers, most rhizobacteria (57%) recorded low IAA production in the range of 1-5 µg ml^{-1}. Around 13% of IAA producers recorded more than 10 µg ml^{-1} of IAA production. Most beneficial bacteria have been reported to produce IAA in this range and were found sufficient to effect root elongation. IAA

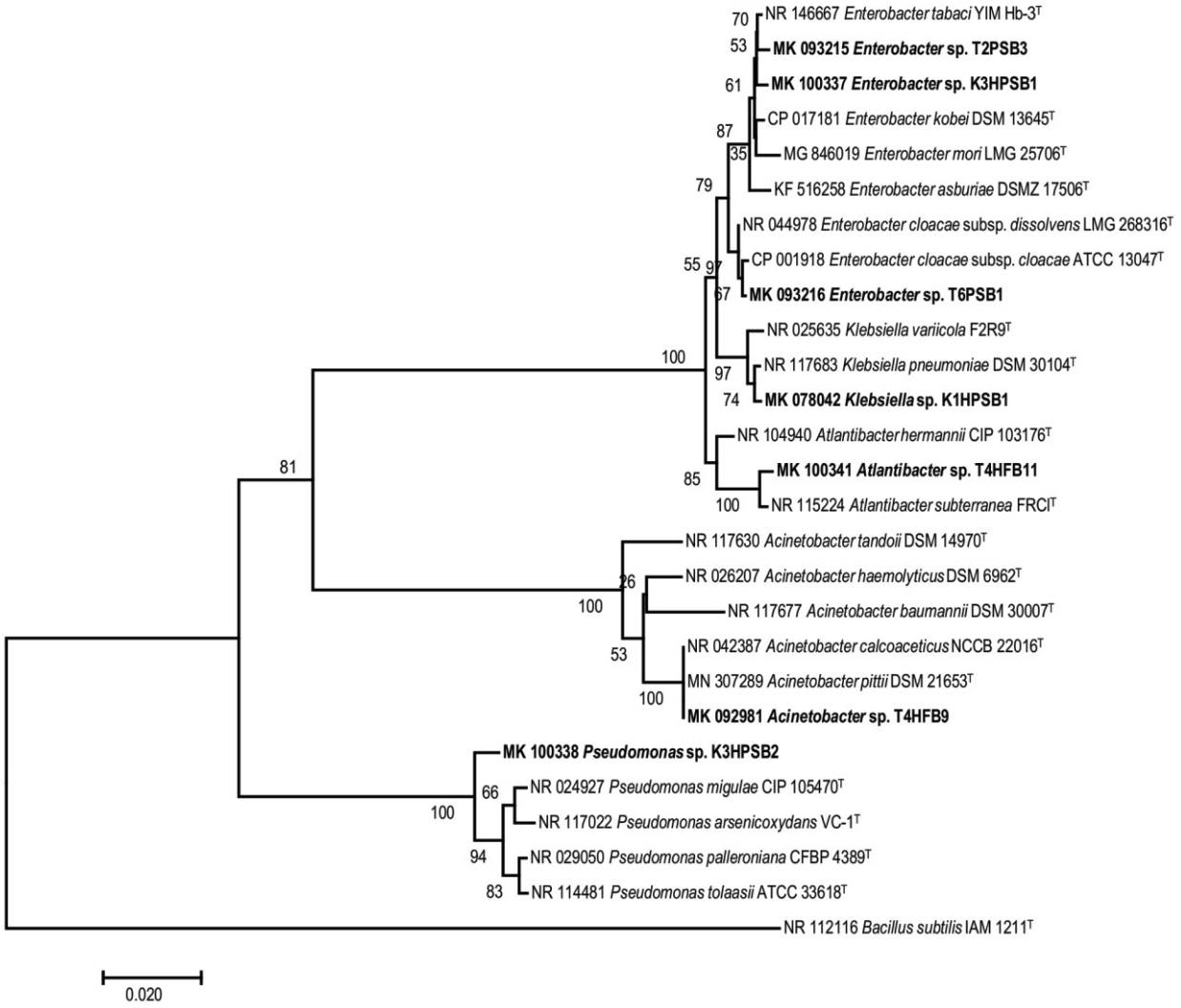

Fig. 5: Phylogram showing genetic relationship among the PGPR strains and their most closely related bacteria obtained from NCBI database.

producing isolates are known to exhibit better colonizing efficiency stimulating over production of root, hairs and lateral roots thus providing the plants increased access of soil nutrients (Qessaoui *et al.,* 2019). Physiological changes effected on coconut roots due to RWD that block nutrient and water uptake remain a big challenge in implementing effective disease management strategies (Muralidharan *et al.,* 1986). Enhancing the fitness of root associated bacterial communities might possibly strengthen the host root system to be responsive to water and nutrient management strategies and to overcome biotic stress.

Screening for mineral nutrient solubilization potential of rhizobacterial isolates was given due importance in this study. Among the nutrient solubilizers, silicate solubilizers were dominant, most of which were capable of solubilizing insoluble phosphate. Out of 110, 57% bacteria solubilized magnesium trisilicate. Phosphate solubilization potential was observed in 48% of the isolates. All the 53 phosphate solubilizers solubilized silicate. Out of 23 Zn solubilizers and 18 potassium solubilizers, most of them solubilized silicate and phosphate (Fig. 1). Seven percent of the tested isolates were able to solubilize all the four insoluble mineral nutrients tested. Earlier investigations in RWD endemic zone revealed acidic nature and poor nutrient status of soil (especially potassium) with less content of exchangeable bases and poor base exchanging capacity. Lower level of magnesium and silica were reported in the leaves of RWD palms (Khan and Krishnakumar, 2018). Higher number of silicate solubilizers in rhizosphere of healthy palms in RWD tract relating it with possibility of higher silica uptake and high foliar silica concentration was reported earlier (Gopal *et al.,* 2005). Functional diversity analyses of microbial communities in citrus rhizosphere have shown abundance of nutrient (nitrogen, carbon and phosphorus) cycling genes in healthy citrus rhizosphere than in ca. *L. asiaticus*-infected citrus (Trivedi *et al.,* 2012). Seven isolates *viz.,* K1HPSB1, K3HPSB1, K3HPSB2, T4HFB9, T4HFB11,

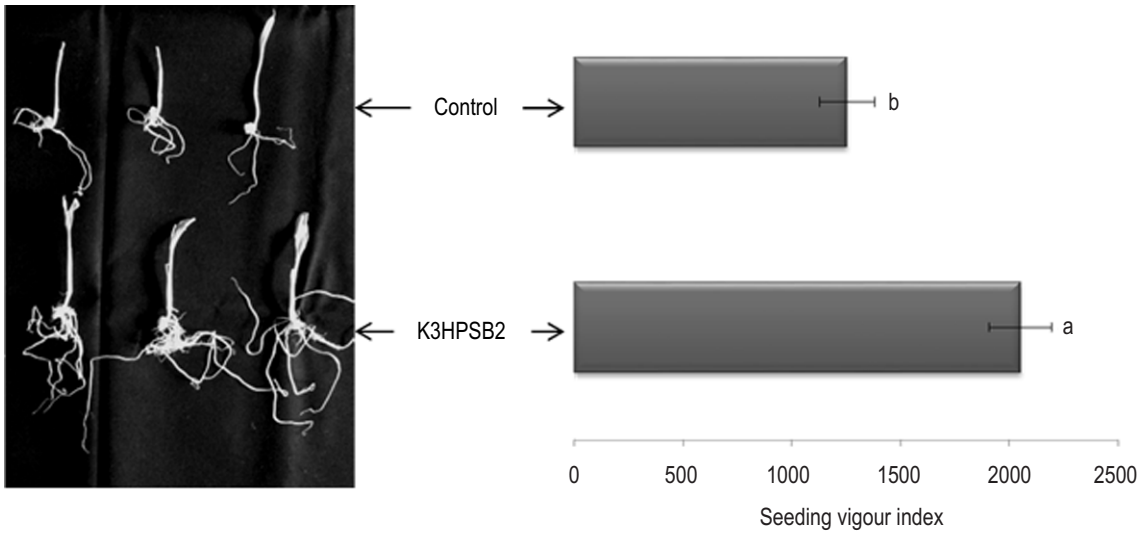

Fig. 6: Effect of *Pseudomonas* sp. strain K3HPSB2 on maize seedling vigour index. Error bar denotes standard error of mean (n = 15). Means with different letters differ significantly at $p < 0.05$.

T2PSB3, T6PSB1 that showed multiple phyto-beneficial properties (IAA production, P, Zn, Si and K solubilization) were selected for further characterization. K1HPSB1 recorded maximum IAA production (20 µg ml^{-1} of culture filtrate) after 72 hr of incubation (Fig. 2). Solubilization indices for tricalcium phosphate and potassium alumino silicate was in the range of 1 to 2 for all the seven isolates tested. Solubilization indices of K3HPSB2 for tricalcium phosphate, magnesium tri silicate and zinc oxide peaked significantly higher than others reaching as high as 1.8, 5.25 and 5, respectively. Solubilization index of K3HPSB2 for potassium (2.0) was on par with that of K1HPSB1 which remained the highest among the tested isolates (Fig. 3, 4). All these isolates grew well in nitrogen-free medium indicating their nitrogen fixing potential. Biochemical characteristics of the isolates are given in Table 2. They were Gram-negative and catalase positive indicating their aerobic nature. Only K3HPSB2 was positive for oxidase test, a key test for differentiating Pseudomonadaceae and Enterobacteriaceae family.

For molecular identification of selected seven isolates, 16S rRNA gene amplification was performed using universal bacterial primers (F27/R1492) that yielded amplicons of ~1465bp size. Sequences of all the seven selected PGPR were deposited in GenBank under unique accession numbers (Table 2). BLASTN searches on the sequences against nucleotide database for sequence homologues revealed the identity of selected rhizobacteria at genus level. All the seven PGPR isolates identified belonged to class Gammaproteobacteria. Though most bacterial plant pathogens came under the class Gammaproteobacteria, majority were harmless or mutualistic and were found in significantly high numbers associated with rhizosphere of healthy host plants when compared to infected and bulk soil (Trivedi *et al.,* 2012). Mendes *et al.* (2011) identified a group of bacterial species belonging to γ-proteobacteria, particularly pseudomonads, consistently associated with *Rhizoctonia solani* disease suppressive soil in sugar beet. The disease suppressive activity of these pseudomonad members was governed by genes expressing non-ribosomal peptide synthetases such as syringomycin-syringopeptin. Phylogenetic tree was constructed with 16S rRNA sequences of bacteria (type species) retrieved from GenBank database that were closely related to the sequences under this study (Fig.5). The analysis involved 27 nucleotide sequences after removing ambiguous positions from each sequence pair. There were a total of 1302 positions in the final dataset. The phylogenetic tree branched into three monophyletic groups in γ-subgroup of proteobacteria. *Bacillus subtilis*, a Gram-positive firmicute was used as an outgroup organism.

Three *Enterobacter* spp. *viz.,* T2PSB3, K3HPSB1, T6PSB1 segregated into two groups with the former two grouping with their closely related type strain *Enterobacter tabaci* strain YIM Hb-3 that was reported as a novel member of the genus isolated from a tobacco stem (Duan *et al.,* 2015) and the latter grouping with *Enterobacter cloacae* strains. Together with K1HPSB1 (closely related to reference strain *Klebsiella pneumoniae* DSM 30104) and T4HFB11 (closely related to reference strain *Atlantibacter subterranea* FRC1), they formed a single cluster representing Enterobacter-Escherichia clade. All these five isolates were Gram-negative, catalase positive and oxidase negative, typical of Enterobacteriales. Isolate T4HFB9 shared the same node with *Acinetobacter pitii* strain ATCC 19004^{T} and *A. calcoaceticus* NCCB 22016^{T} in a separate cluster. It was non-motile, Gram-negative coccus with catalase positive and oxidase negative activity.

Several rhizospheric and endophytic Enterobacteriales including *Enterobacter, Serratia, Klebsiella* spp. and *Acinetobacter* spp. known to be human pathogens were also reported to exhibit PGP traits and were found to enhance vegetative growth and yield (Rokhbakhsh-Zamin *et al.,* 2011; Gupta *et al.,* 2014; Bhardwaj *et al.,* 2017). Selection of phyto-beneficial rhizobacteria for bioinoculant applications strictly follow 'Environmental and Human Safety Index' protocol (Vilchez, 2016). *Pseudomonas* is one of the bacterial genera extensively exploited as plant growth promoting rhizobacterium. The green fluorescent *Pseudomonas* K3HPSB2, isolated during this study, showed high sequence similarity (98.64%) with *Pseudomonas migulae* CIP 105470^T, which was reported from natural mineral waters in France (Verhille *et al.,* 1999). However, the 16S rRNA tree topology revealed that it formed a separate branch from closely related strains of genus *Pseudomonas viz., Pseudomonas migulae* CIP 105470^T, *P. arsenicoxydans* VC-1^T, *Pseudomonas palleroniana* CFBP 4389^T and *Pseudomonas tolaasii* ATCC 33618^T (Fig. 5).

Early seedling vigour is an imperative trait correlated positively with general plant health and stress tolerance. Plant-beneficial rhizobacterium interaction has evidenced reduced pathogenic infection by promoting plant growth and activating disease defensive pathways (Lakkis *et al.,* 2019). Our results on short term effect of biopriming in maize seeds with *Pseudomonas* sp. K3HPSB2 indicated enhanced seedling vigour. Germination percentage was significantly higher with rhizobacterial treatment (93) over control (60). Analysis of variance revealed significant increase in number of roots along with root and shoot length in PGPR inoculated seedlings thereby recording higher seedling vigour index (1811.60) against the control (751.80) (Fig. 6). Growth stimulating effects of *Pseudomonas* spp. has been evidenced in several crops (Sekar *et al.,* 2018; Qessaoui *et al.,* 2019) attributing to different mechanisms such as production of IAA, antifungal metabolites, siderophores and hydrolytic enzymes and solubilzation of phosphates. Disease alleviating potential of *Pseudomonas* spp. against phytoplasmal infection (Gamelero *et al.*, 2010; 2017) and other plant diseases (Sekar *et al.,* 2018; Lakkis *et al.,* 2019) is also worth mentioning in this context.

Plant-microbiome interaction strongly influences the health status of the host along with functional stability of associated microbial communities. This work describes preliminary attempts to decipher the functional (phytobeneficial) aspects of culturable rhizosphere bacterial diversity associated with naturally evolved root wilt diseased resistance of coconut palms in diseased hot spot areas.

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Add-on Information

Authors' contribution: S. Indhuja: conceived, planned and carried out the experiments; analyzed results and wrote the manuscript; **M. Babu:** Molecular characterization of rhizobacteria; **A. Gupta:** Devised the project, the main conceptual ideas and proof outline; **M. Gopal:** contributed to result interpretation and the final version of the manuscript; **J. Mathew:** Soil analysis; **R.J. Thomas:** Selection of mother palms, facilitated sampling and correction of manuscript; **A.A. Harıs:** Supervised the work and proofreading manuscript; **V. Krishnakumar:** Facilitated research implementation, critical feedback and helped in manuscript editing.

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